

We claim:

1. A method for producing a 4-cyano-3-hydroxybutyric acid ester from a 4-halo-3-hydroxybutyric acid ester, the method comprising:

(a) providing a 4-halo-3-hydroxybutyric acid ester,

5 wherein the halo substituent is selected from the group consisting of chlorine, bromine, and iodine; and

(b) contacting the 4-halo-3-hydroxybutyric acid ester with a halohydrin dehalogenase and cyanide under conditions sufficient to form a reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester.

10 2. The method of claim 1, wherein the 4-cyano-3-hydroxybutyric acid ester is a non-racemic chiral 4-cyano-3-hydroxybutyric acid ester.

3. The method of claim 1, wherein the cyanide is provided by hydrocyanic acid.

15 4. The method of claim 1, wherein the cyanide is provided by a cyanide salt.

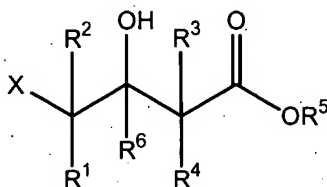
5. The method of claim 1, wherein the halo substituent of the 4-halo-3-hydroxybutyric acid ester is selected from chlorine and bromine.

20 6. The method of claim 1, wherein the 4-halo-3-hydroxybutyric acid ester is a 4-chloro-3-hydroxybutyric acid ester.

25 7. The method of claim 1, wherein the 4-halo-3-hydroxybutyric acid ester is a lower alkyl ester.

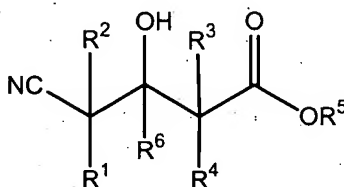
8. The method of claim 1, wherein

(1) the 4-halo-3-hydroxybutyric acid ester has the structure:



and

(2) the 4-cyano-3-hydroxybutyric acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

$R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ , and  $R^6$  are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and

$R^5$  is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl.

9. The method of claim 1, wherein the halohydrin dehalogenase is a naturally occurring halohydrin dehalogenase.

10. The method of claim 1, wherein the halohydrin dehalogenase is a non-naturally occurring halohydrin dehalogenase.

11. The method of claim 1, wherein the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester is maintained at a pH in the range of from about 5 to about 9.

12. The method of claim 11, wherein the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester is maintained at a pH in the range of from about 5 to about 8.

13. The method of claim 1, wherein the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester is maintained at a pH of about 8 or below.

14. The method of claim 1, wherein the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester further comprises a pH buffer.

15. The method of claim 1, further comprising:  
(c) adding a base sufficient to maintain the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester at a pH of about 5 or above.

16. The method of claim 15 wherein the base is selected from hydroxide salts, carbonate salts, and bicarbonate salts.

17. The method of claim 15 wherein the base is selected from a cyanide salt.

18. The method of claim 1, further comprising recovering the 4-cyano-3-hydroxybutyric acid ester from the reaction mixture for converting the 4-halo-3-hydroxybutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester.

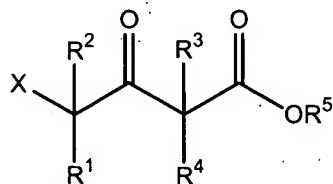
19. The method of claim 16, further comprising purifying the 4-cyano-3-hydroxybutyric acid ester.
- 5 20. The method of claim 1, step (a) comprises providing a 4-halo-3-ketobutyric acid ester, wherein the halo substituent is selected from the group consisting of chlorine, bromine, and iodine; and contacting the 4-halo-3-ketobutyric acid ester with a ketoreductase, a cofactor, and a  
10 cofactor regeneration system under conditions sufficient to form a reaction mixture for converting the 4-halo-3-ketobutyric acid ester to the 4-halo-3-hydroxybutyric acid ester.
21. The method of claim 20, wherein the cofactor is NAD/NADH.
- 15 22. The method of claim 20, wherein the cofactor is NADP/NADPH.
23. The method of claim 20, wherein the ketoreductase is a naturally occurring ketoreductase.
- 20 24. The method of claim 20, wherein the ketoreductase is a non-naturally occurring ketoreductase.
- 25 25. The method of claim 20, wherein the cofactor regeneration system comprises glucose and a glucose dehydrogenase.
26. The method of claim 25, wherein the glucose dehydrogenase is a naturally occurring glucose dehydrogenase.
27. The method of claim 25, wherein the glucose dehydrogenase is a non-naturally  
30 occurring glucose dehydrogenase.

28. The method of claim 20, wherein the cofactor regeneration system comprises formate and a formate dehydrogenase.

29. The method of claim 28, wherein the formate dehydrogenase is a naturally occurring formate dehydrogenase.

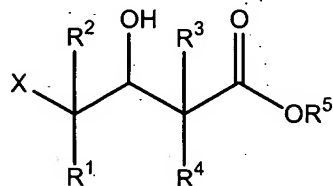
30. The method of claim 28, wherein the formate dehydrogenase is a non-naturally occurring formate dehydrogenase.

31. The method of claim 20, wherein  
(1) the 4-halo-3-ketobutyric acid ester has the structure:



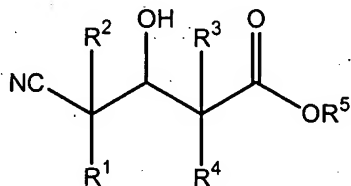
and

(2) the 4-halo-3-hydroxybutyric acid ester has the structure:



and

(3) the 4-cyano-3-hydroxybutyric acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

$R^1$ ,  $R^2$ ,  $R^3$ , and  $R^4$  are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and

$R^5$  is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl.

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32. The method of claim 20, wherein the reaction mixture for converting the 4-halo-3-ketobutyric acid ester to the 4-halo-3-hydroxybutyric acid ester is maintained at a pH in the range of from about 5 to about 10.

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33. The method of claim 20, wherein the reaction mixture for converting the 4-halo-3-ketobutyric acid ester to the 4-halo-3-hydroxybutyric acid ester further comprises a buffer.

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34. The method of claim 25, further comprising:  
adding a base sufficient to maintain the reaction mixture for converting the 4-halo-3-ketobutyric acid ester to the 4-halo-3-hydroxybutyric acid ester at a pH of about 5 or above.

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35. A method for producing a 4-cyano-3-hydroxybutyric acid ester from a 4-halo-3-ketobutyric acid ester, the method comprising:

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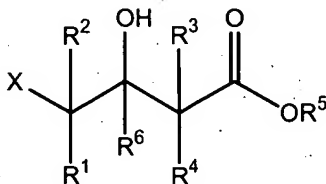
- (a) providing a 4-halo-3-ketobutyric acid ester,  
wherein the halo substituent is selected from the group consisting of chlorine, bromine, and iodine; and
- (b) contacting the 4-halo-3-ketobutyric acid ester with a ketoreductase, a cofactor, a cofactor regeneration system, cyanide, and a halohydrin dehalogenase to form a reaction mixture for converting the 4-halo-3-ketobutyric acid ester to a 4-cyano-3-hydroxybutyric acid ester.

36. A method for producing a 4-nucleophile substituted-3-hydroxybutyric acid ester or amide from a 4-halo-3-hydroxybutyric acid ester or amide, the method comprising:

- (a) providing a 4-halo-3-hydroxybutyric acid ester or amide,  
wherein the halo substituent is selected from the group consisting of chlorine,  
bromine, and iodine; and
- (b) contacting the 4-halo-3-hydroxybutyric acid ester or amide with a halohydrin  
dehalogenase and a nucleophile under conditions suitable to form a reaction mixture for  
converting the 4-halo-3-hydroxybutyric acid ester or amide to a 4-nucleophile substituted-3-  
hydroxybutyric acid or amide.

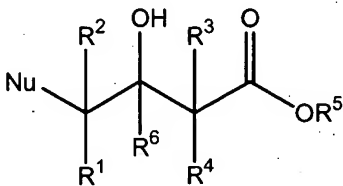
37. The method of claim 36, wherein

- (1) the 4-halo-3-hydroxybutyric acid ester or amide is a 4-halo-3-hydroxybutyric acid  
ester having the structure:



and

- (2) the 4-nucleophile substituted-3-hydroxybutyric acid ester or amide is a 4-nucleophile  
substituted-3-hydroxybutyric acid ester having the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, and R<sup>6</sup> are each independently selected from the group consisting of  
hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl,  
an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally  
substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally

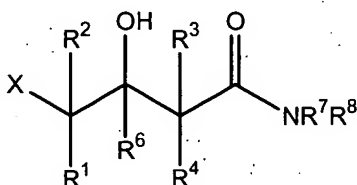
substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, and an optionally substituted aryloxy, an optionally substituted arylalkoxy; and

$R^5$  is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl.; and

Nu is selected from the group consisting of  $-\text{CN}$ ,  $-\text{N}_3$ , and  $-\text{ONO}$ .

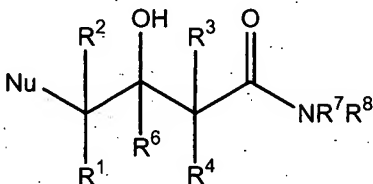
38. The method of claim 36, wherein

(1) the 4-halo-3-hydroxybutyric acid ester or amide is a 4-halo-3-hydroxybutyric acid amide having the structure:



and

(2) the 4-nucleophile substituted-3-hydroxybutyric acid ester or amide is a 4-nucleophile substituted-3-hydroxybutyric acid ester having the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;  
 $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ , and  $R^6$  are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and



$R^7$  and  $R^8$  are each independently selected from the group consisting of hydrogen, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl; and

Nu is selected from the group consisting of  $-CN$ ,  $-N_3$ , and  $-ONO$ .

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39. The method of claim 36, wherein step (a) comprises:

providing a 4-halo-3-ketobutyric acid ester or amide,

wherein the halo substituent is selected from the group consisting of chlorine, bromine, and iodine; and

10

contacting the 4-halo-3-ketobutyric acid ester or amide with a ketoreductase, a cofactor, and a cofactor regeneration system under conditions suitable to form a reaction mixture for converting the 4-halo-3-ketobutyric acid ester or amide to the 4-halo-3-hydroxybutyric acid ester or amide.

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40. A method for producing a 4-nucleophile substituted-3-hydroxybutyric acid esters or amide, the method comprising:

(a) providing a 4-halo-3-ketobutyric acid ester or amide

wherein the halo substituent is selected from the group consisting of chlorine, bromine, and iodine; and

20

(b) contacting the 4-halo-3-ketobutyric acid ester or amide with a ketoreductase, a cofactor, a cofactor regeneration system, a nucleophile, and a halohydrin dehalogenase to form a reaction mixture for converting the 4-halo-3-ketobutyric acid ester or amide to a 4-nucleophile substituted-3-hydroxybutyric acid ester or amide.

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41. A composition comprising:

(a) a halohydrin dehalogenase;

(b) a nucleophile; and

(c) a 4-halo-3-hydroxybutyric acid ester or amide.

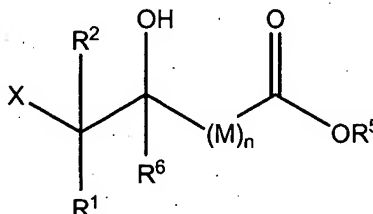
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42. The composition of claim 41, wherein the nucleophile is cyanide.

43. A method for producing a vicinal cyano, hydroxy substituted carboxylic acid ester from a vicinal halo, hydroxy substituted carboxylic acid ester, the method comprising:
- (a) providing a vicinal halo, hydroxy substituted carboxylic acid ester,  
wherein the halo substituent is selected from the group consisting of chlorine,  
5 bromine, and iodine; and
- (b) contacting the vicinal halo, hydroxy substituted carboxylic acid ester with a  
halohydrin dehalogenase and cyanide under conditions suitable to form a reaction mixture for  
converting the vicinal halo, hydroxy substituted carboxylic acid ester to a vicinal cyano,  
hydroxy substituted carboxylic acid ester.
- 10
44. The method of claim 43, wherein the vicinal cyano, hydroxy substituted carboxylic acid ester is a non-racemic chiral vicinal cyano, hydroxy substituted carboxylic acid ester.
- 15
45. The method of claim 43, wherein the cyanide is provided by hydrocyanic acid.
46. The method of claim 43, wherein the cyanide is provided by a cyanide salt.
47. The method of claim 43, wherein the halo substituent of the vicinal halo,  
20 hydroxy substituted carboxylic acid ester is selected from chlorine and bromine.
48. The method of claim 43, wherein the vicinal halo, hydroxy substituted carboxylic acid ester is a vicinal chloro, hydroxy substituted carboxylic acid ester.
- 25
49. The method of claim 43, wherein the vicinal halo, hydroxy substituted carboxylic acid ester is a lower alkyl ester.

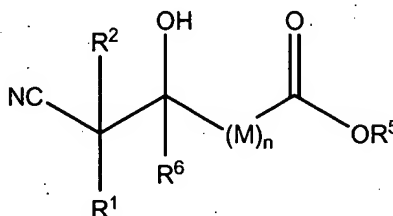
50. The method of claim 43, wherein

- (1) the vicinal halo, hydroxy substituted carboxylic acid ester has the structure:



and

- 5 (2) the vicinal cyano, hydroxy substituted carboxylic acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

10 R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and

15 R<sup>5</sup> is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl; and.

20 each M<sub>n</sub> is independently selected from -C(=O)- (i.e., carbonyl) or -CR<sup>n</sup>R<sup>m</sup>-, wherein R<sup>n</sup> and R<sup>m</sup> are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, an optionally substituted aryl, hydroxyl, nitro, amino, cyano, carboxy

(i.e. a carboxylate or carboxylic acid group), carboalkoxy (i.e. an ester group), carbamide (i.e. an amide group), and acyl (i.e. forming a ketone); and

n is zero or an integer from 1 to 9, inclusive.

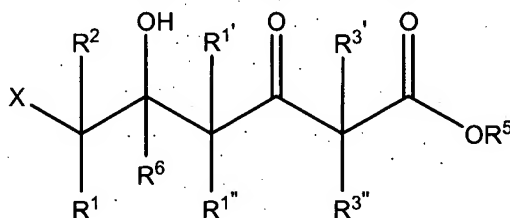
5 51. The method of claim 50, wherein n is an integer from 2 to 8, inclusive.

52. The method of claim 50, wherein n is 3.

10 53. The method of claim 43, wherein the vicinal cyano, hydroxy substituted carboxylic acid ester is a 6-halo-5-hydroxyhexanoic acid ester.

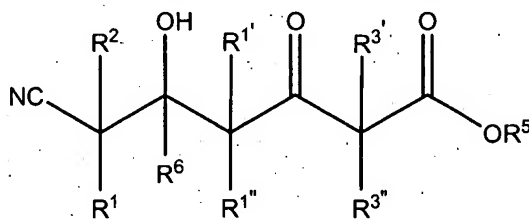
54. The method of claim 43, wherein

(1) the vicinal halo, hydroxy substituted carboxylic acid ester has the structure:



15 and

(2) the vicinal cyano, hydroxy substituted carboxylic acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

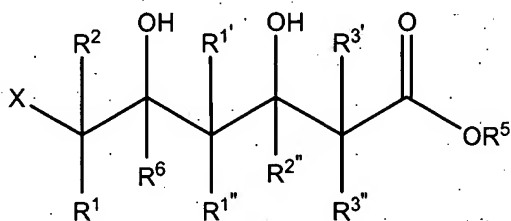
20 R<sup>1</sup>, R<sup>2</sup>, R<sup>3</sup>, R<sup>4</sup>, R<sup>6</sup>, R<sup>1'</sup>, R<sup>1''</sup>, R<sup>3'</sup>, and R<sup>3''</sup> are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally

substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and

$R^5$  is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl.

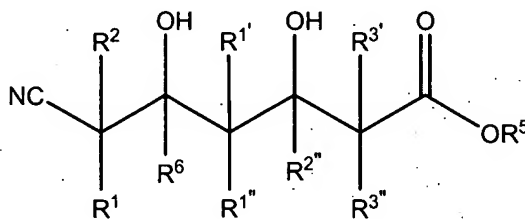
55. The method of claim 43, wherein

(1) the vicinal halo, hydroxy substituted carboxylic acid ester has the structure:



10 and

(2) the vicinal cyano, hydroxy substituted carboxylic acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

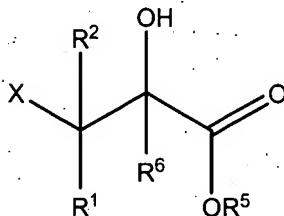
15  $R^1$ ,  $R^2$ ,  $R^3$ ,  $R^4$ ,  $R^6$ ,  $R^1$ ,  $R^{1''}$ ,  $R^{2''}$ ,  $R^{3'}$ , and  $R^{3''}$  are each independently selected from the group consisting of hydrogen, fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and

$R^5$  is selected from the group consisting of an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted arylalkyl.

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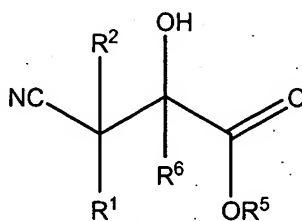
56. The method of claim 43, wherein

- (1) the vicinal halo, hydroxy substituted carboxylic acid ester has the structure:



and

- 5 (2) the vicinal cyano, hydroxy substituted carboxylic acid ester has the structure:



wherein:

X is a halogen selected from the group consisting of chlorine, bromine, and iodine;

- R<sup>1</sup>, R<sup>2</sup>, and R<sup>6</sup> are each independently selected from the group consisting of hydrogen,  
10 fluorine, an optionally substituted lower alkyl, an optionally substituted cycloalkyl, an  
optionally substituted lower alkenyl, an optionally substituted aryl, an optionally substituted  
arylalkyl, amino, an optionally substituted lower alkylamino, an optionally substituted  
cycloalkylamino, an optionally substituted lower alkoxy, an optionally substituted  
cycloalkoxy, an optionally substituted aryloxy, and an optionally substituted arylalkoxy; and  
15 R<sup>5</sup> is selected from the group consisting of an optionally substituted lower alkyl, an  
optionally substituted cycloalkyl, an optionally substituted aryl, and an optionally substituted  
arylalkyl.

57. The method of claim 43, wherein the halohydrin dehalogenase is a naturally  
20 occurring halohydrin dehalogenase.

58. The method of claim 43, wherein the halohydrin dehalogenase is a non-naturally occurring halohydrin dehalogenase.

59. The method of claim 43, wherein the reaction mixture for converting the vicinal halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester is maintained at a pH in the range of from about 5 to about 9.
- 5 60. The method of claim 59, wherein the reaction mixture for converting the vicinal halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester is maintained at a pH in the range of from about 5 to about 8.
61. The method of claim 43, wherein the reaction mixture for converting the  
10 vicinal halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester is maintained at a pH of about 8 or below.
62. The method of claim 43, wherein the reaction mixture for converting the  
15 vicinal halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester further comprises a pH buffer.
63. The method of claim 43, further comprising:  
(c) adding a base sufficient to maintain the reaction mixture for converting the vicinal  
20 halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester at a pH of about 5 or above.
64. The method of claim 63 wherein the base is selected from hydroxide salts, carbonate salts, and bicarbonate salts.
- 25 65. The method of claim 63 wherein the base is selected from a cyanide salt.
66. The method of claim 43, further comprising recovering the vicinal cyano, hydroxy substituted carboxylic acid ester from the reaction mixture for converting the vicinal  
30 halo, hydroxy substituted carboxylic acid ester to a vicinal cyano, hydroxy substituted carboxylic acid ester.

67. The method of claim 64, further comprising purifying the vicinal cyano, hydroxy substituted carboxylic acid ester.

68. A composition comprising:

- 5 (a) a halohydrin dehalogenase;
- (b) cyanide; and
- (c) a vicinal halo, hydroxy substituted carboxylic acid ester.